REMARKS

Priority Claim:

The specification has been amended to correct an error that was noted in the priority claim as submitted with the Preliminary Amendment upon filing this application. Specifically, the priority claim should have recited that U.S. Patent Application Serial No. 07/962,522 is a continuation-in-part (not a continuation) of U.S. Patent Application Serial No. 07/911,760. Also, the priority claim has been amended to a format that is believed to be preferred by the Examiner.

New Claims:

Claims 48-54 have been added to more particularly describe the present invention. Support for support for Claim 48 is found on page 10, lines 21-25; support for Claim 49 is found on page 10, lines 21-25; support for Claim 50 is found on page 10, lines 13-16; support for Claims 51 and 52 is found on page 10, lines 1-7; and support for Claims 53 and 54 is found on page 9, lines 20-23.

Objection to the Specification and Rejection of Claims 38-47 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has rejected Claims 38-47, contending that there does not appear to be support in the specification for a product by process of growing microflora in a culture medium containing less than about 3 grams of chloride per liter of the culture medium, sources of carbon, nitrogen, micronutrients, and a non-chloride sodium salt at a temperature from about 5°C to about 48°C.

The Examiner's rejection of Claims 38-47 under 35 U.S.C. § 112, first paragraph is respectfully traversed. Initially, it is noted that the phrasing in Claim 38 has been reorganized in an attempt to clarify the claim language. Support for the phrasing in Claim 38 is found in the specification as follows. First, working examples showing the production of a biomass comprising the microflora using the process recited in Claim 38 is exemplified by Examples 13, 15 and 16, wherein a biomass is produced by culturing microflora of the present invention at a temperature of between about 5°C and about 48°C, in a culture medium comprising: (1) less than about 3 grams of chloride per liter of culture medium; (2) sources of carbon, nitrogen and nutrients; and (3) a non-chloride sodium salt. Further, support for the provision of less than about 3 grams of chloride per liter of said culture medium is found, for example, on page 9, lines 1-7. Support for the use of a non-

chloride sodium salt is found, for example, on page 10, line 13 to page 11, line 3. Support for the growth at a temperature between about 5°C and about 48°C is found, for example, on page 12, lines 17-20. Support for growth of microflora in a culture medium including sources of carbon, nitrogen and micronutrients is found throughout the examples, and particularly on pages 42-44 (Example 11). Since the term "nutrients" is used in the specification to describe additional nutrients added to culture medium, however (e.g., see page 38, line 16), this term has been substituted for the term "micronutrients" in Claim 38.

In view of the foregoing discussion, the Examiner is respectfully requested to withdraw the rejection of Claims 38-47 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 38 and 41-47 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has rejected Claims 38 and 41-47 Under 35 U.S.C. § 112, first paragraph, contending that the specification, while enabling for members of the order Thraustochytriales, which are *Thraustochytrium* and *Schizochytrium*, does not reasonably provide enablement for any and all species of the order Thraustochytriales. The Examiner asserts that although the order does not have many species, the specification does not provide support for any additional members beyond *Thraustochytrium* and *Schizochytrium* and mixtures thereof.

The rejection of Claims 38 and 41-47 under 35 U.S.C. § 112, first paragraph is respectfully traversed. It is submitted that the invention was never intended to be limited to *Thraustochytrium* and *Schizochytrium*; rather, *Thraustochytrium* and *Schizochytrium* are merely examples of suitable microorganisms for use in the production of the claimed biomass. It is not only part of the invention, but it is also just as easy to isolate other members of the order Thraustochytriales as those exemplified in the specification.

The enablement requirement refers to the requirement of 35 U.S.C. 112, first paragraph that the specification describe how to make and how to use the invention. The invention that one skilled in the art must be enabled to make and use is that defined by the claims. The claimed invention is enabled if one skilled in the art can make and use the invention without undue or unreasonable experimentation.

First, it is noted that, in the paragraph bridging pages 8 and 9 of the present application, a collection and screening process is described. Page 9, lines 2-3 of the present application states: "This process is described in detail in related U.S. Patent No. 5,130,242." The present application has now been amended to include the detailed description of this collection and screening process for other euryhaline microorganisms, which includes microorganisms of the order Thraustochytriales from U.S. Patent No. 5,130,242 (see the amendment to the specification set forth above), which was incorporated by reference in the present application. Additionally, a specific embodiment of a collection and screening technique is described in great detail in Example 1 on page 20 of the present application. The collection and screening process fully enables one skilled in the art to practice the full scope of the claimed invention and to obtain other microorganisms of the order Thraustochytriales in addition to microorganisms of the genera *Thraustochytrium*, *Schizochytrium* and mixtures thereof. It is respectfully submitted that the specification sets forth a repeatable method by which such microorganisms are obtainable.

Specifically, the screening process outlined in the present application is an important advance in the art. To someone experienced in screening (i.e., the skilled artisan), the collection and screening method is easy to follow without undue experimentation. Importantly, multiple strains can be screened on a single substrate simultaneously. The desired strains are easily identified on the substrate. The screening method taught in the present invention is designed to accomplish a number of steps simultaneously and easily (e.g., screen for size, color, heterotrophic growth, euryhalinity, thermal tolerance). More linear methods known in the art prior to the invention could require a great deal of experimentation. But the method disclosed in the present application screens, in essentially one step, a large number of strains simultaneously for a number of desirable characteristics. Only a few screenings were needed to obtain suitable microorganisms. Indeed, the Examiner has acknowledged that the order Thraustochytriales contains few members, which further supports the position herein that one of skill in the art can readily identify and collect other members of the order without undue experimentation. Further, it is noted that in related Application Serial No. 09/461,709, now U.S. Patent No. 6,451,567, the Examiner acknowledged that this specification was enabling for the scope of growing euryhaline microorganisms, which is a broader scope than what is set forth in the present claims.

In *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) the court ruled that undue experimentation would not be required to practice an invention directed to monoclonal antibody technology. The court found that the specification provided considerable direction and guidance on how to practice the claimed invention and presented working examples, that all of the methods needed to practice in the invention were well known, and that there was a high level of skill in the art at the time the application was filed. The same three criteria also apply in the present case. Therefore, that present specification meets the enablement requirement.

The Examiner has not identified or put forth any rationale as to why the collection and screening method would not work to identify other suitable microorganisms within this order. As set forth in Section 2164.04 of the MPEP, in the absence of such an explanation by the Examiner, the reasonable assertion by the Applicant that the collection and screening method enables one to isolate microorganisms meeting the full scope of the claimed invention should be accepted. Therefore, the specification enables one skilled in the art to which it pertains to obtain other Thraustochytriales microorganisms for use in the claimed invention.

In view of the foregoing discussion, the Examiner is respectfully requested to withdraw the rejection of Claims 38 and 41-47 under 35 U.S.C. § 112, first paragraph.

Rejection of Claim 40 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has rejected Claim 40 under 35 U.S.C. § 112, first paragraph, contending that it is not clear that the recited microorganism is readily available to the public.

To address this rejection, enclosed herewith is a Declaration of the agent of record which will satisfy the requirement under 35 U.S.C. 112, first paragraph.

In view of the foregoing discussion, the Examiner is respectfully requested to withdraw the rejection of Claim 40 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 38-47 Under 35 U.S.C. § 112, Second Paragraph:

The Examiner has rejected Claims 38-47 under 35 U.S.C. § 112, second paragraph, contending that Claims 38-47 are rendered vague and indefinite because the recited product by process claim allegedly does not appear to be well supported in the specification. In addition, the Examiner contends that Claims 39-47 are vague and indefinite for the recitation of "aquaculture feed

composition" and "said primary source of sodium ion", both of which allegedly lack antecedent basis in the amended independent claim.

Claims 38-47 are believed to be definite with regard to the recitation of the product by process for the reasons set forth in the discussion above under 35 U.S.C. § 112, first paragraph.

Claims 39-47 have been amended in a manner consistent with the Examiner's suggestions to provide the proper antecedent basis for the phrases in the dependent claims.

In view of the foregoing discussion, the Examiner is respectfully requested to withdraw the rejection of Claims 38-47 under 35 U.S.C. § 112, second paragraph.

Objection to Claim 42 Under 37 CFR § 1.75(c):

The Examiner has objected to Claim 42 under 37 CFR § 1.75(c), contending that the claim fails to further limit the subject matter of a previous claim.

Claim 42 has been canceled without prejudice to or disclaimer of the subject matter therein. In view of the foregoing discussion, the Examiner is respectfully requested to withdraw the objection to Claim 42 under 37 CFR § 1.75(c).

It is respectfully submitted that all claims are in condition for allowance, and the Examiner is respectfully requested to pass this application to issue. In the event the Examiner does not allow all claims or has any questions regarding the claims, please consider this an invitation to contact the undersigned agent at (303) 863-9700 to discuss the remaining concerns.

Respectfully submitted,

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Date: Nov. 4, 2002

Marked Version Showing Amendments

In the Specification:

The paragraph on page 1, line 5 has been amended as follows:

--Cross-Reference to Related Applications

This application is a divisional of U.S. Patent Application Serial No. 09/270,301, filed March 15, 1999, [which issued as] now U.S. Patent No. 6,103,225[on August 15, 2000], which is a divisional of U.S. Patent Application Serial No. 08/842,874, filed April 17, 1997, [which issued as] now U.S. Patent No. 5,908,622 [on June 1, 1999], which is a continuation of U.S. Patent Application Serial No. 08/461,137, filed June 5, 1995, [which issued as] now U.S. Patent No. 5,688,500 [on November 18, 1997], which is a divisional of U.S. Patent Application Serial No. 08/292,490, filed August 18, 1994, [which issued as] now U.S. Patent No. 5,518,918 [on May 21, 1996], which is a divisional of U.S. Patent Application Serial No. 07/962,522, filed October 16, 1992, [which issued as] now U.S. Patent No. 5,340,742 [on August 23, 1994], which is a [continuation] continuation-in-part of U.S. Patent Application Serial No. 07/911,760, filed July 10, 1992, [which issued as] now U.S. Patent No. 5,340,594 [on August 23, 1994], which is a divisional of U.S. Patent Application Serial No. 07/580,778, filed September 11, 1990, [which issued as] now U.S. Patent No. 5,130,242 [on July 14, 1992], which is a continuation-in-part of U.S. Patent Application Serial No. 07/439,093, filed November 17, 1989, now abandoned, which is a continuation-in-part of U.S. Patent Application Serial No. 07/241,410, filed September 7, 1988, now abandoned, disclosures of which are incorporated by reference herein in their entirety.--

On page 9, line 4, the following new paragraphs have been added:

--Collection, isolation and selection of large numbers of suitable heterotrophic strains can be accomplished by the following method. Suitable water samples and organisms typically can be collected from shallow, saline habitats which preferably undergo a wide range of temperature and salinity variation. These habitats include marine tide pools, estuaries and inland saline ponds, springs, playas and lakes. Specific examples of these collection sites are: 1) saline warm springs such as those located along the Colorado river in Glenwood Springs, Colo., or along the western edge of the Stansbury Mountains, Utah; 2) playas such as Goshen playa located near Goshen, Utah; 3)

marine tide pools such as those located in the Bird Rocks area of La Jolla, Calif.; and 4) estuaries, such as Tiajuana estuary, San Diego County, Calif. Special effort should be made to include some of the living plant matter and naturally occurring detritus (decaying plant and animal matter) along with the water sample. The sample can then be refrigerated until return to the laboratory. Sampling error is minimized if the water sample is shaken for 15-30 seconds, prior to pipetting or pouring a portion, for example, 1-10 ml, into a filter unit. The filter unit includes 2 types of filters: 1) on top, a sterile Whatman #4 filter (Trademark, Whatman Inc., Clifton, N.J.); and 2) underneath the Whatman filter, a polycarbonate filter with 1.0 μm pore size. The purpose of the first (top) filter is to remove all particulate matter greater than about 25 μm, generally allowing only unicellular type material to pass onto the 1.0 μm polycarbonate filter. The first filter greatly reduces the number of mold colonies that subsequently develop upon incubation of the polycarbonate filter at elevated temperatures, thereby enhancing the opportunities for other colonies to develop. Mold spores are very numerous in coastal and inland saline waters, and mold colonies can quickly cover an agar plate unless screened out. The 1.0 μm size of the polycarbonate filter is chosen to allow many of the bacteria to pass on through into the filtrate. The purpose of using a sandwich filter design is to select for unicellular organisms at least a portion of whose cells range in diameter from about 1 μm to about 25 μm in size (organisms which could potentially be grown easily in a fermentor system for production on a large scale). Extensive growth of these unicellular organisms can be encouraged by incubation of the polycarbonate filter on an agar plate. Competition between organisms growing on the filter facilitates the isolation of competitive, robust strains of single-celled microorganisms. Unicellular aquatic microorganisms selected by the foregoing method display a range of cell size depending on growth conditions and stage of reproductive cycle. Most cells in culture have diameters in the range from about 1 μm to about 25 μm; however, cells (thalli and sporangia) in the cultures can be found that have larger diameters (depending on the strain) up to about 60 μm.

After filtration, the polycarbonate filter can be placed on an agar plate containing saline media containing a source of organic carbon such as carbohydrate including glucose, various starches, molasses, ground corn and the like, a source of assimilable organic or inorganic nitrogen such as nitrate, urea, ammonium salts, amino acids, microbial growth factors included in one or more of yeast extract, vitamins, and corn steep liquor, a source of assimilable organic or inorganic

phosphorous, and a pH buffer such as bicarbonate. Microbial growth factors are currently unspecified compounds which enhance heterotrophic growth of unicellular microorganisms, including fungi and algae. The agar plates can be incubated in the dark at 25°-35°C. (30°C is preferred) and after 2-4 days numerous colonies will have appeared on the filter. Recovery of 1-5 colonies/plate of the desired organism is not uncommon. Yeast colonies are distinguishable either by color (they frequently are pink) or by their morphology. Yeast colonies are smooth whereas the desired organisms form in colonies with rough or textured surfaces. Individual cells of the desired organism can be seen through a dissecting microscope at the colony borders, whereas yeast cells are not distinguishable, due to their smaller size. Mold and higher fungi colonies are distinguishable from the desired organisms because they are filamentous, whereas the desired organisms are non-filamentous. Clear or white-colored colonies can be picked from the plates and restreaked on a new plate of similar media composition. While most of the desired organisms are clear or white-colored, some are orange or red-colored due to the presence of xanthophyll pigments and are also suitable for selection and restreaking. The new plate can be incubated under similar conditions, preferably at 30°C. and single colonies picked after a 2-4 day incubation period. Single colonies can then be picked and placed in, for example, 50 ml of liquid medium containing the same organic enrichments (minus agar) as in the agar plates. These cultures can be incubated for 2-4 days at 30°C with aeration, for example, on a rotary shaker table (100-200 rpm.). When the cultures appear to reach maximal density, 20-40 ml of the culture can then be harvested by centrifugation or other suitable method and preserved, as by lyophilization. The sample can then be analyzed by standard, well-known techniques including gas chromatography techniques to identify the fatty acid content of the strain. Those strains with omega-3 highly unsaturated fatty acids can thereby be identified and cultures of these strains maintained for further screening.

Promising strains can be screened for temperature tolerance by inoculating the strains into 250 ml shaker flasks containing 50 ml of culture media. These cultures are then incubated for 2 days on the shaker table over any desired temperature range from most practically between 27°-48°C, one culture at each 3°C interval. Production can be quantified as the total amount of fatty acids produced per ml of culture medium. Total fatty acids can be quantified by gas chromatography as described above. A similar process can also be employed to screen for salinity tolerance. For salinity tolerance

a range of salinities yielding conductivities from 5-40 mmho/cm is adequate for most purposes. Screening for the ability to utilize a variety of carbon and nitrogen sources can also be conducted employing the procedure outlined above. The carbon and nitrogen sources were evaluated herein at concentrations of 5 g/l. Carbon sources evaluated were: glucose, corn starch, ground corn, potato starch, wheat starch, and molasses. Nitrogen sources evaluated were: nitrate, urea, ammonium, amino acids, protein hydrolysate, corn steep liquor, tryptone, peptone, or casein. Other carbon and nitrogen sources can be used, the choice being open to those of ordinary skill in the art, based on criteria of significance to the user.--

In the Claims:

Claim 42 has been canceled.

Claims 48-54 have been added.

Claims 38-41 and 42-47 have been amended as follows:

38. (Twice Amended) A biomass comprising microflora selected from the order Thraustochytriales, wherein said microflora is produced by a process comprising growing said microflora at a temperature from about 5°C to about 48°C in a culture medium containing: less than about 3 grams of chloride per liter of said culture medium[,]; sources of carbon, nitrogen, and nutrients [micronutrients,]; and a non-chloride sodium salt[at a temperature from about 5°C to about 48°C].

39. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein said microflora is selected from the group consisting of *Thraustochytrium*, *Schizochytrium*, and mixtures thereof.

40. (Once Amended) The [aquaculture feed composition] biomass of Claim [77]39, wherein said *Thraustochytrium*, *Schizochytrium*, and mixtures thereof, have all of the identifying characteristics of an organism selected from the group consisting of ATCC Nos. 20888, 20889, 20890, 20891, and 20892, and mutants thereof, wherein said mutants have an omega-3 highly unsaturated fatty acid content of at least about 0.5% dry weight.

41. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein [said] the primary source of said non-chloride sodium [ion] salt is sodium sulfate.

43. (Once Amended) The [aquaculture feed composition] biomass of Claim [79]41, wherein a concentration of said sodium sulfate in said culture medium is between about 6 g/l and about 309 g/l.

44. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein said microflora have a sterol content of at least about 0.1% ash-free dry weight.

45. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein said microflora have a cholesterol content of at least about 15% of the total sterol content.

46. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein said microflora have a cell aggregate size less than about 100 microns.

47. (Once Amended) The [aquaculture feed composition] biomass of Claim [76]38, wherein said microflora have a cell aggregate size less than about 50 microns.